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ORIGINAL ARTICLE **OPEN ACCESS**

Determination of Tenofovir by HPLC, UV Visible Spectrophotometer and InfraRed Spectroscopy

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ABSTRACT
HIV is a virus, when administered into the body it destroys T cells. HIV can be spread through Blood, Semen, vaginal and rectal fluids. Purpose of Current study was to determine Tenofovir (an anti-viral drug) by HPLC, UV Visible Spectrophometer and Infra-red Spectroscopy. For UV visible spectrophotometric study, Shimadzu 1800 double beam UV visible spectrophotometer, UV probes 2.33 was utilized. Tenofovir drug was identified by IR spectroscopy and also by Melting point determination and solubility. HPLC study of Tenofovir Alafenamide was also performed. The accuracy of drug was determined at three levels 50%, 100% and 150%. The % drug recovered were 98.50%, 101.42% and 99.63% respectively. During study of Intraday Precision, % RSD for Tenofovir Alafenamide was found in range of 0.17--0.49. LOD and LOQ of Tenofovir Alafenamide also determined and were found to be 0.40 µg/ml and 1.23 µg/ml respectively. The validated method was simple, precise, accurate and reproducible and therefore suitable for routine analysis of drugs in tablet dosage form.
Keywords: *Tenofovir, HPLC, UV Spectrophotometer, IR Spectroscopy, Accuracy, Precision.*

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INTRODUCTION

Tenofovir Alafinamide is an Anti-viral Drug. Tenofovir alafenamide is used to treat different viral infections like HIV and infections of liver i.e. chronic hepatitis B infection. Tenofovir Alafinamide works by stopping or slowing the growth of the virus. Chronic hepatitis infection can lead to serious liver damage (cirrhosis) and liver cancer. Tenofovir alafenamide belongs to a group of HIV drugs called nucleoside reverse transcriptase inhibitors (NRTIs) [1-4]. HIV is a virus that enters your body and begins to destroy T cells. You need T cells in order to fight infections. HIV spreads through bodily fluids that include:Blood, semen, vaginal and rectal fluids, and breast milk. The first few weeks after infection is called the acute infection stage. During this time the virus rapidly reproduces. Your immune system responds by producing HIV antibodies [6]. Many people experience temporary flu-like symptoms during this stage. Even without symptoms, HIV is highly contagious during this time. Some people infected with HIV are asymptomatic at first. Most people experience symptoms in the first month or two after becoming infected. That's because your immune system is reacting to the virus as it rapidly reproduces. This early stage is called acute stage [5]. Symptoms are similar to those of the flu and may last anywhere from a few days to several weeks. These include: Fever, swollen lymph glands, general aches and pains. During the first few months of infection, an HIV test may provide a false-negative result. This is because it takes time for the immune system to build up enough antibodies to be detected in a blood test. But the virus is active and highly contagious during this time. The clinical latent infection, or chronic stage of HIV, can last from a few years to a few decades. During this time the virus is still reproducing, but at lower levels. Some people have few, if any, symptoms. Others may have many symptoms. Without antiretroviral therapy, you're likely to pass through this phase faster. Several analytical methods that have been reported for the individual determination of TE in biological fluids and pharmaceutical formulations which include liquid chromatography coupled with spectrofluorimetric and mass spectroscopy detection.

Objectives of Current Study:

a) To identify the Drug (Tenofovir Alafinamide) by IR Spectroscopy, Melting Point and Solubility.
b) To determine the Drug (Tenofovir Alafinamide) by UV Spectrophotometer.
c) To determine the Drug (Tenofovir Alafinamide) by HPLC by using different Parameters.

MATERIAL AND METHODS

Tenofovir Alafenamide and other chemicals were obtained from Yash Pharmaceuticals. UV Visible spectrophotometer (Shimadzu 1800 double beam UV visible spectrophotometer, UV probe 2.33) was used for analysis. Melting point apparatus used is of Chemi Line.

RESULTS AND DISCUSSION

Identification of Drugs

Determination of Solubility [7]:

Solubility of Tenofovir Alafenamide was determined in different solvents and final solubility of Drug is given in Table .1.

Table1 : Determination of Solubility

Drug	Solubility
Tenofovir Alafenamide	Slightly soluble in water and sparingly soluble in Methanol

Determination of Melting Point:

Table 2: Determination of Melting Point:

Drug	Observed	StdMeltimg point
TenofovirAlafenamide	199-202 ^{0}C	198-205 ^{0}C

Identification by IR [8]:

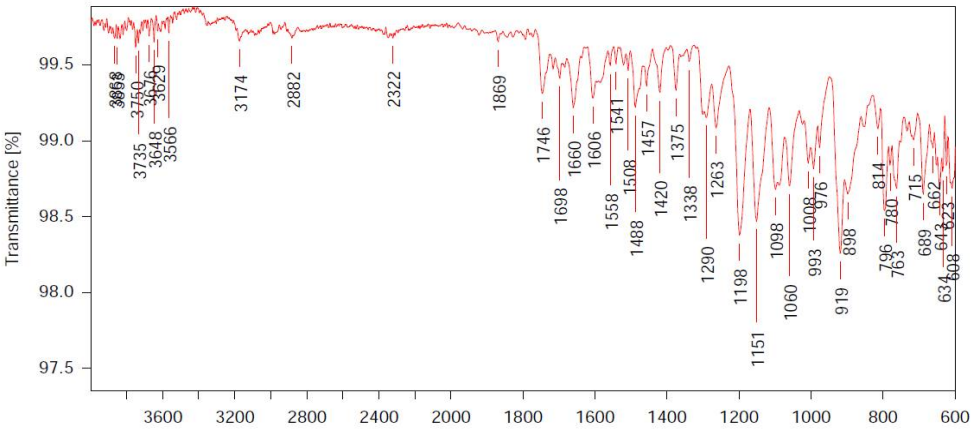

Figure 1: IR spectra of sample

Functional Group	Wave number
C-N	1290
P=O	1198
C=O	1660
N-H	1541

Selection of wavelength [9]

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of TenofovirAlafenamide (10 µg/ml) was prepared in Methanol. This drug solution was than scanned in UV region of 190-400 nm and maximum Absorbance was recorded.

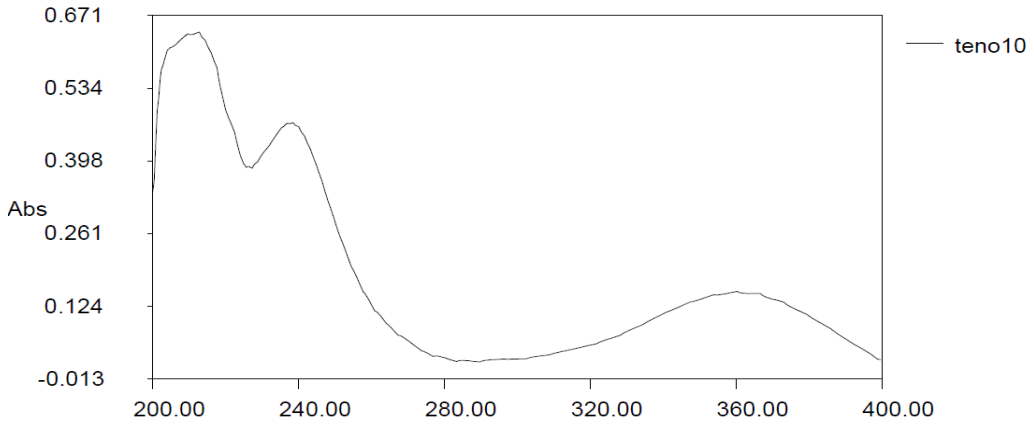

Figure 2: UV Spectra of Tenofovir Alafenamide(10 µg/ml) (Maximum Absorbance 237 nm)

Tenofovir Alafenamide solution: 10 mg-→100 ml with DIluent. Further 1 ml to a 10 ml and make up with Diluent (10µg/ml). Solution was scanned between 190 - 400 nm.

Wavelength What Gives maximum Absorbance was selected from the above Spectra.

HPLC Study [10]:

Table 3:Instrumentation for HPLC

Component	Brand / Model / Software
HPLC	1200 series/ Agilent,
HPLC Column	Inertsil ODS- 3v (250*4.6mm)
Detector	UV detector
Ultrasonicator	Frontline machinery
Digital pH meter	Analab
Analytical Balance	Shimadzu

Method Validation:

Accuracy:

For Tenofovir Alafenamide

The accuracy of drug was determined at three levels 50%, 100% and 150% [6]. The area of each solution peak was measured at 285 nm wavelength. The amount of Tenofovir Alafenamide was calculated at each level and % recoveries were computed.

Table 4: Recovery data for TenofovirAlafenamide

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Average Recovered	% RSD
1	50 %	5	4	4.014	98.50	1.75
2		5	4	3.877		
3		5	4	3.930		
4	100 %	5	5	5.035	101.42	1.57
5		5	5	5.134		
6		5	5	5.195		
7	150 %	5	6	6.036	99.63	0.84
8		5	6	5.951		
9		5	6	5.948		

Intraday precision

In Intraday precision,% RSD for Tenofovir Alafenamide was found in range of 0.17--0.49 [7].

Table 5: Intraday precision data for TenofovirAlafenamide

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
1	5	958.39±2.21	0.23
2	10	1898.68±9.30	0.49
3	15	2874.19±5.10	0.17

LOD and LOQ [8]:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 9: LOD and LOQ data for Tenofovir Alafenamide

LOD	LOQ
LOD = 3.3 x (SD / Slope) = 3.3 x (23.45/190.31) = **0.40 µg/ml**	LOQ = 10 x (SD / Slope) = 10x (23.45/190.31) = **1.23 µg/ml**

RESULT AND DISCUSSION

The identification and HPLC study of Tenofovir Alafenamide was carried out. For identification study, initially Solubility and melting point was determined. Further, it is identified by Infrared spectroscopy. Detection wavelength was determined by UV visible spectrophotometer. HPLC Method validation was performed by using different parameters like Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ). For determination of Accuracy, drug was tested at three different levels i.e 50, 100 and 150%. The % drug recovered was 98.50%, 101.42% and 99.63% respectively. In Intraday precision, % RSD for Tenofovir Alafenamide was found to be 0.23, 0.49 and 0.17. LOD and LOQ of Tenofovir Alafenamide were found to be 0.40 µg/ml and 1.23µg/ml respectively.

CONCLUSION

Tenofovir Alafenamide is an Anti-viral drug. Purpose of current research is to identify and determine the Tenofovir Alafenamide drug. Identification was carried out firstly by Infra-red spectroscopy, then determination of melting point and solubility of Clofarabine. Further, UV visible spectrophotometric study was carried out to optimize detection wavelength, which is beneficial for High performance liquid chromatographic study also. Hence, Tenofovir Alafenamide was scanned between 200-400 nm in UV Visible spectrophotometer.

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